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MODELLING OF GENETICALLY ENGINEERED MICRO-ORGANISMS INTRODUCTION IN CLOSED ARTIFICIAL MICROCOSMS

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ABSTRACT

The possibility of introducing genetically engineered microorganisms (GEM) into simple biotic cycles of laboratory water microcosms was investigated. The survival of the recombinant strain *Escherichia coli* Z905 (Ap^r, Lux^+) in microcosms depends on the type of model ecosystems. During the absence of algae blooming in the model ecosystem, the part of plasmid-containing cells *E.coli* decreased fast, and the structure of the plasmid was also modified. In conditions of algae blooming (*Ankistrodesmus sp.*) an almost total maintenance of plasmid-containing cells was observed in *E.coli* population. A mathematics model of GEM's behavior in water ecosystems with different level of complexity has been formulated. Mechanisms causing the difference in luminescent exhibition of different species are discussed, and attempts are made to forecast the GEM's behavior in water ecosystems.

THE PROBLEM OF GEM'S INTRODUCTION INTO NATURAL ECOSYSTEMS

Genetic engineering is a wide sphere of activities, which includes ecologically controlled and uncontrolled methods of new microorganisms creation (Pechurkin *et al.*, 1990). When introduced in nature the transformed microorganisms may cause ecological disorder, as they are unpeculiar to the natural ecosystems and may lead to unusual competition with the natural microorganism's association because of their enhanced growth characteristics (Smit *et al.*, 1992; Wagner-Dobler *et al.*, 1992).

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SMALL EXPERIMENTAL MODEL CLOSED ECOSYSTEMS

A model system which is used for prognosis needs to demonstrate the structure changes in natural ecosystem, as revealed by the introduction of an alien biological object and its consequences in terms of mobility of recombinant molecule and its fragments within nature. Our Small Experimental Model Closed EcoSystems represent a water microcosms with directed trophical structure, supporting different levels of biogenic and abiogenic loading. The biota of these microcosms include: bacteria of different species (*Micrococcus*, *Arthrobacter*, *Cyanobacter*, *Pseudomonas*, *Bacillus* and *E. coli* Z905- (Ap^r, Lux^+), also introduced), yeasts, protozoa, green algae and microalgae (*Ankistrodesmus* sp.), and also higher organisms Snails, *Daphnia pulex* and fishes *(Lebistes reticulata)*. Different number of species and trophic links of association may be settled, presenting different types of water ecosystems.

GENETICALLY ENGINEERED MICROORGANISMS (GEM)

As a GEM, we have used the strain *Escherichia coli* Z905, containing the recombinant plasmid pPHL (Ap^r, Lux^+), carrying the genes of the luminescent system of the luminous bacteria *Photobacterium leiognathi*, cloned in the vector pUC18 (Illarionov, Protopopova, 1986).

MATHEMATICAL MODELLING OF GEM'S INTRODUCTION

Several variations of GEMs can be included in the trophic chain. In our case the recombinant strain becomes part of the reducer's link achievning waste mineralization. The mathematical model provided below, describes the GEMs dynamics after their introduction into microcosms.

$$dP/dt = \mu_p P - \mu_{c1} C_1 / Y_{c1} - \varepsilon_p P \,, \qquad \text{(Eq.1)}$$

$$dC_1/dt = \mu_{c1} C_1 - \mu_{c2} C_2 / Y_{c2} - \varepsilon_{c1} C_1 \,, \qquad \text{(Eq.2)}$$

$$dC_2/dt = \mu_{c2}C_2 - \varepsilon_{c2}C_2 , \quad \text{(Eq.3)}$$

$$dD/dt = (\varepsilon_p P + \varepsilon_{c1}C_1 + \varepsilon_{c2}C_2 + \varepsilon_R R) - \mu_R R/Y_R , \quad \text{(Eq.4)}$$

$$dR/dt = \mu_R R - \varepsilon_R R , \quad \text{(Eq.5)}$$

$$dN/dt = k_R\mu_R R/Y_R + k_1\mu_{c1}C_1/Y_{c1} + k_2\mu_{c2}C_2/Y_{c2} - \mu_p P/Y_p , \quad \text{(Eq.6)}$$

where X: biomass of producers, Y: biomass of primary consumers, Y: biomass of secondary consumers, R - biomass of reducers (GEM), D: detritus biomass, N: free nitrogen, μ_p, μ_{c1}, μ_{c2}, μ_R: specific growth rates of producers, primary and secondary consumers, reducers (GEM), respectively, ε_p, ε_{c1}, ε_{c2}, ε_R: specific rates of mortality of producers, primary and secondary consumers, reducers (GEM), respectively, Y_p: producers yield coefficient, Y_{c1}: primary consumers yield coefficient, Y_{c2}: secondary consumers yield coefficient, Y_R: reducers yield coefficient, k_i: positive constants.

POPULATION DYNAMICS OF GEM *ESCHERICHIA COLI* Z905 (pPHL7) IN MODEL MICROCOSMS

In this investigation we paid a special attention to the comparison of recombinant strains behavior under conditions of water blooming (Figures 1) and it's absence (Figure 2).
In the experiments, we studied the dynamics of the cells of the recombinant *E.coli* Z905 strain (Ap^r, Lux^+) after it's introduction into microcosms in conditions of water blooming, i.e. with a number of green unicellular algae exceeding 10^6 cells/ml (Figure 1).

The dynamics of the ecosystem's microflora is shown on Figure 1. After inoculation of the cells containing recombinant plasmid, changes in microbial populations of the microcosm took place.

However the number of introduced recombinant cells *E.coli* remained practically constant throughout the experiment. Without "blooming", the level of unicellular algae did not exceed 10^2 cells/ml. The dynamics of the different variants inside the recombinant strain E.coli Z905 population are shown on Fig. 2.

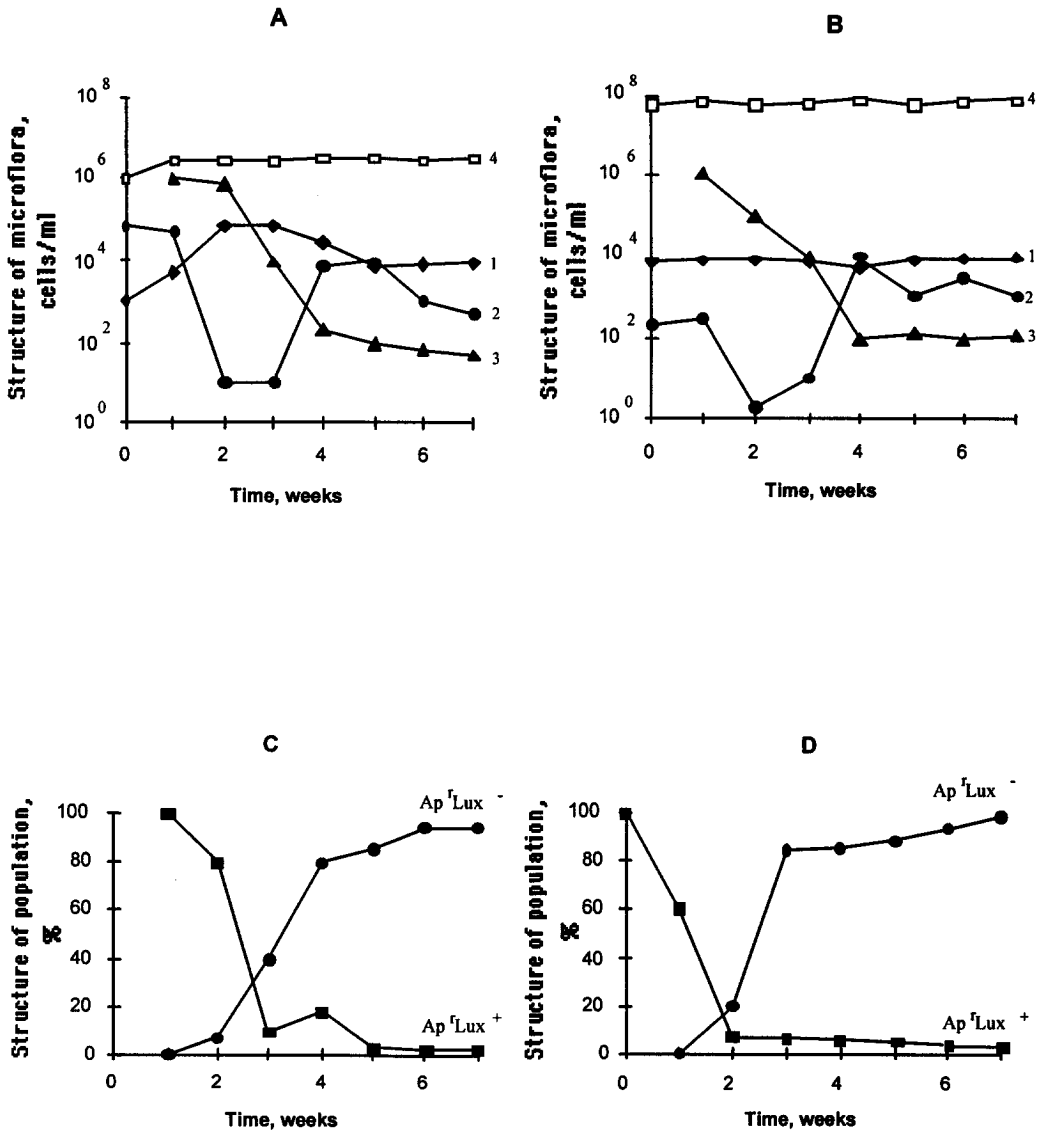


Figure 1. The dynamics of the ecosystem's microflora (A, B) and different variants *E. coli* Z905 population (C, D) after introduction in microcosm in conditions of the green algae "blooming".

(A, C): volume of water in the microcosm is 20 l, (B, D): it is 4 l.

1: yeast, 2: bacteria, 3: E.coli, 4: microalgae.

The resulting population consisted of three cells types: non-plasmid (Ap^S, Lux^-), cells containing vector plasmid (Ap^r, Lux^-), cells of the initial strain containing the whole plasmid (Ap^r, Lux^+). Thus, in a microcosm without blooming the population of the recombinant strain E.coli is successfully introduced in the heterotrophic link of the ecosystem microflora.

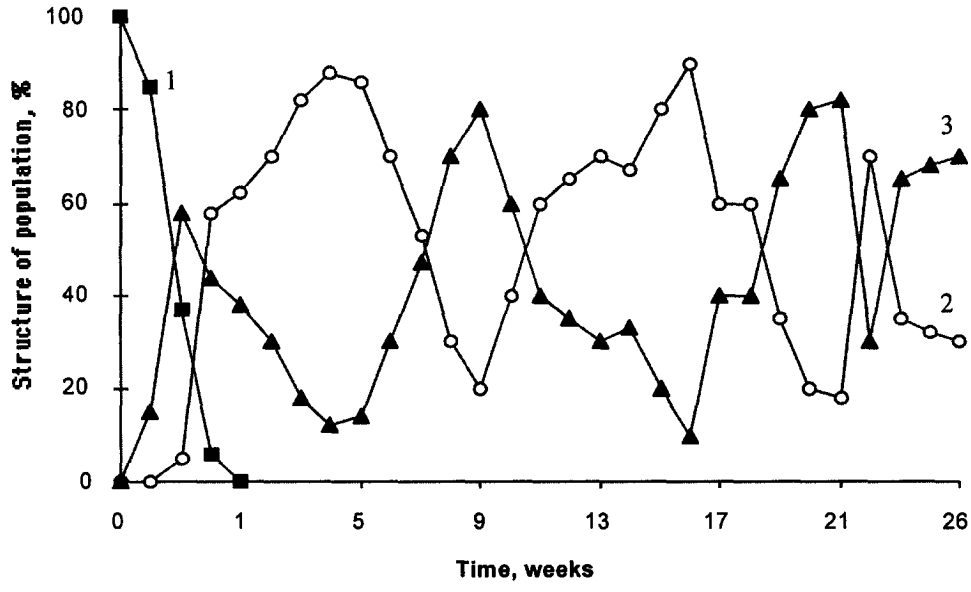
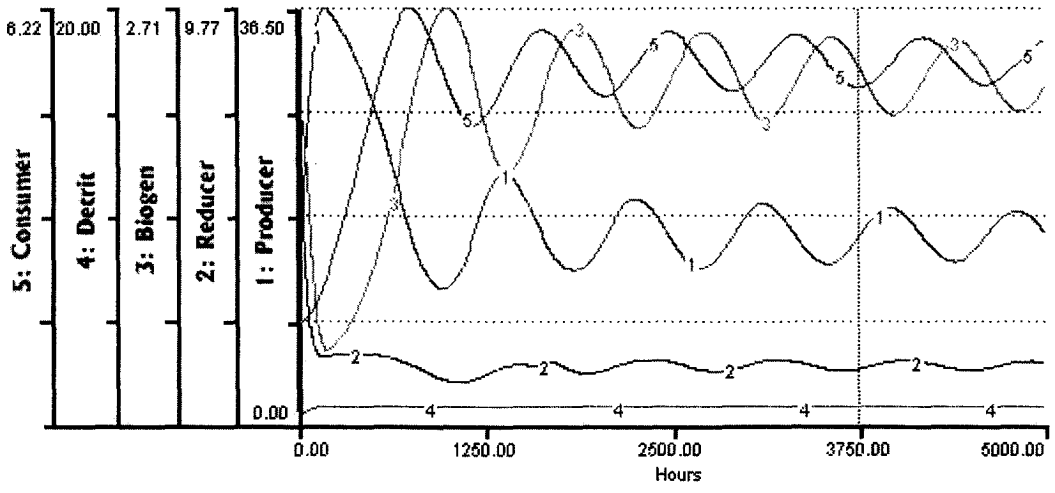


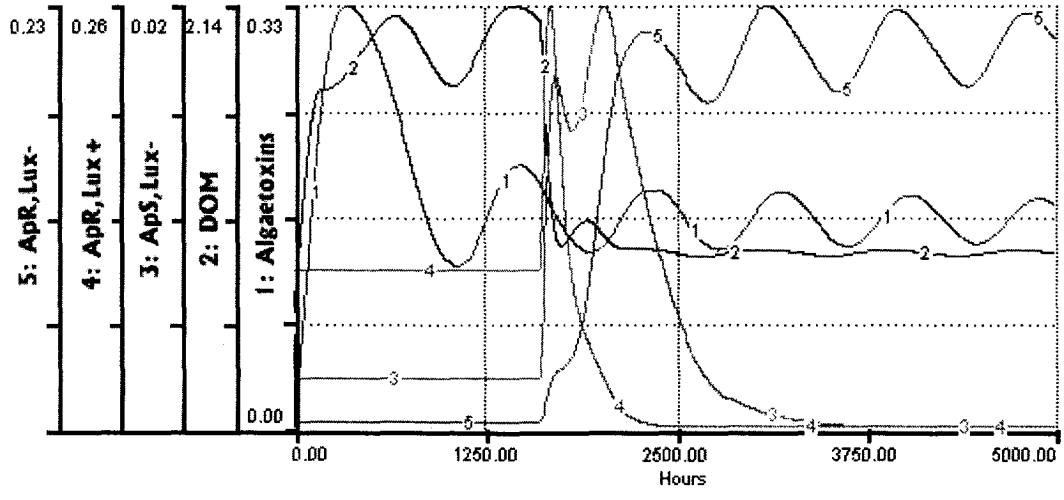
Figure 2. The dynamics of and different variants E. coli Z905 population after introduction in microcosm in conditions without "blooming".

1: (Ap^r, Lux $^+$), 2: (Ap^r, Lux $^-$), 3: (Ap^S, Lux $^-$).

In contrast to the experiments without water blooming (Figure 2), there was no more non-plasmid cells with (Ap^S, Lux $^-$) phenotype in conditions of water blooming (Figures 1). Such a difference in *E.coli* recombinant strain populations structure under the different blooming conditions may be caused by the different concentrations of the toxic materials accumulated in the water as a result of the green unicellular algae's growth. Thus the recombinant strain inoculated in these microcosms is viable and may remain obviously for a long time. The results of mathematical modelling are in a good agreement with experimental data (Figure 3).



1: Producer, 2: Reducer, 3: Biogen, 4: Detrit, 5: Consumer



1: Algaetoxins, 2: DOM, 3: $Ap^{s}lux^{-}$, 4: $Ap^{r}Lux^{+}$, 5: $Ap^{r}Lux^{-}$

Figure 3. The biomass dynamics (mg/l) of the trophic chains (A) and different variants E.coli Z905 population (B) after introduction in microcosm in conditions of the green algae "blooming" according to mathematical model (eq. 1:6).

CONCLUSIONS

1. The *E.coli* recombinant strain population is rather competitive with heterotrophic microorganisms in water laboratory microcosms, a feature which leads to it's successful inclusion in microcosm's trophic chains as a heterotrophic link.

2. Under the different levels of abiotic factors, both during, and in absence of blooming *E.coli* cells containing the recombinant plasmid (the whole or having lost the cloned fragment) remained in the microcosm's microflora at least for 7-20 weeks.

3. During all the experiments we did not observe the plasmid transfer to other species of microcosm's microflora. However, this may be due to the low bacteria concentration.

4. The laboratory microcosms allow an evaluation the feasibility of introducing a recombinant strain into trophic chains of an ecosystem, an assessment of their competitiveness and viability, and an observation of the ability of recombinant plasmid to be transferred to the natural strains, and to express the cloned genes in different conditions.

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